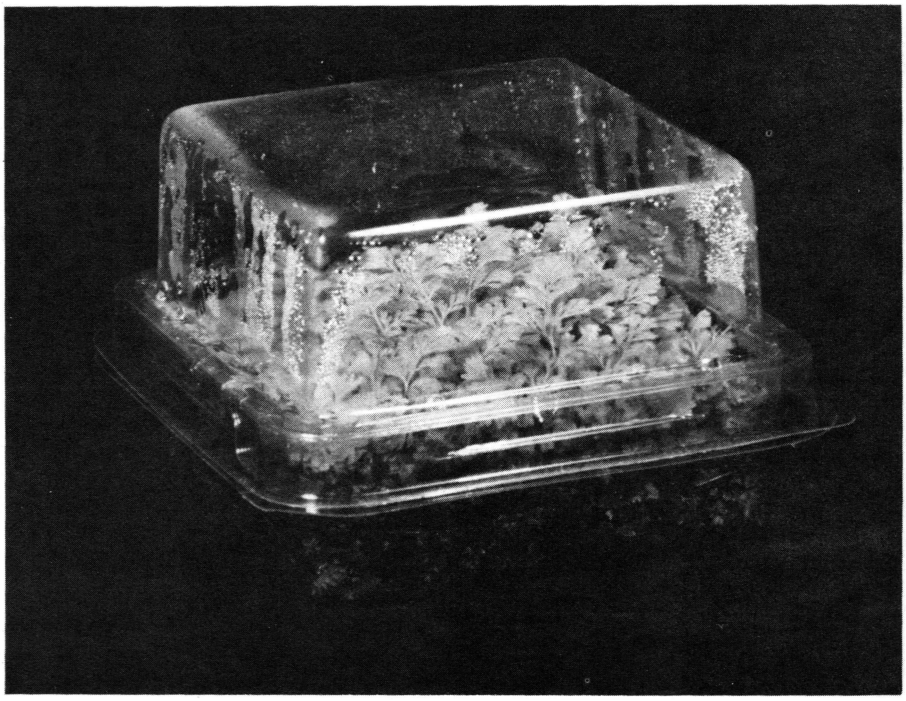

Fig. 2. *Davallia* multiple growth after homogenization

removed in 2 weeks. The survival rate for both plants was about 80%.

The number of plantlets produced in the containers by this process is comparable to standard methods utilizing manual dissection and transfer of plantlets, however, it eliminates labor intensive work and thus may save on operating costs of the laboratory. In addition, the reduction of many manual transfers may decrease contamination spread by contact.

This method 1) employs a single medium for all steps, 2) eliminates many laborious transfers and 3) carries the potential to be mechanized on a large scale. It remains to determine which species currently being propagated by tissue culture methods can be adapted to subculture by mechanical blending.

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In Vitro Propagation of *Cucumis sativus* L.[1]

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Abstract. **Axillary buds of gynoecious cucumber (*Cucumis sativus* L.) were cultured on a semi-solid medium containing Murashige and Skoog salts plus thiamin, pyridoxin, nicotinic acid, myo-inositol, and sucrose with 0.1 mg/liter naphthaleneacetic acid (NAA) and 0.1 mg/liter kinetin. Both shoots and roots were proliferated with little callus formation. Plants produced *in vitro* were successfully transferred to soil.**

Gynoecius hybrids are being used in increasing amounts in commercial cucumber production and a large percentage of pickling cucumber cultivars are gynoecious hybrids (10). In order to develop gynoecious inbreds for use in a gynoecious hybrid breeding program, single selections must be vegetatively propagated to provide enough plants for inbreeding. The current practice involves rooting 3 or 4 cuttings in aerated water containing indolebutyric acid (1,2,3). Gibberellic acid is then used to induce production of staminate flowers on at least 1 plant to furnish pollen for propagation of the gynoecious selection. In some cases, however, plants will fail to root or will be lost, particularly when transferring to soil. This becomes especially significant when only 3 or 4 cuttings can be obtained from 1 plant and at least 2 (1 staminate and 1 pistillate) must survive to maintain the selection. A tissue culture method of propagation that could produce numerous plants from a single gynoecious selection would be desirable. Such a procedure has not been previously reported for cucumbers although shoot regeneration from callus of pumpkin has been reported (4,5). Maciejewska-Potapczykowa et al. (7) succeeded in initiating callus from cucumber stem fragments and floral buds but their main interest was the study of hormonal effects on sex expression rather than propagation.

In the early part of this investigation attempts were made to initiate callus from explants. Callus formation occurred readily on stem pieces of the cultivar 'Carolina' cultured on a medium containing Murashige and Skoog salts (9) and the following organic additives in mg/liter; thiamin, 0.4; pyridoxin, 1.0; nicotinic acid, 5.0; myo-inositol, 100; sucrose, 30,000; and agar, 7000; with NAA, 0.1; and kinetin, 1.0. The use of callus culture was abandoned, however, because no organ regeneration occurred after varying the concentrations of NAA and kinetin in the nutrient medium.

A propagation procedure using axillary buds was developed which avoided callus formation. Axillary buds of 'Carolina' were excised from 1 month old plants grown in a growth chamber at 27°C (day) and 19°C (night) temperature, under a 12 hr photoperiod at 32.3 klx. Each leaf axil was surface-sterilized in a 2.5% sodium hypochlorite solution for 2 min and then rinsed in sterile deionized water. Axillary buds measuring 1-3 mm long were removed with the aid of a dissecting microscope. Explants were placed into culture on a medium based on Murashige and Skoog salts (9) plus the same organic additives as used for callus with various levels of NAA (0-0.5 mg/liter) and kinetin (0-20.0 mg/liter). Twelve axillary buds per treatment were used. The pH was adjusted to 5.7 with 0.1N NaOH prior to sterilization at 121°C for 15 min. Cultures were incubated at 25°C in a growth chamber with 16 hr photoperiod at 4.3 klx provided by cool white fluorescent tubes and incandescent bulbs.

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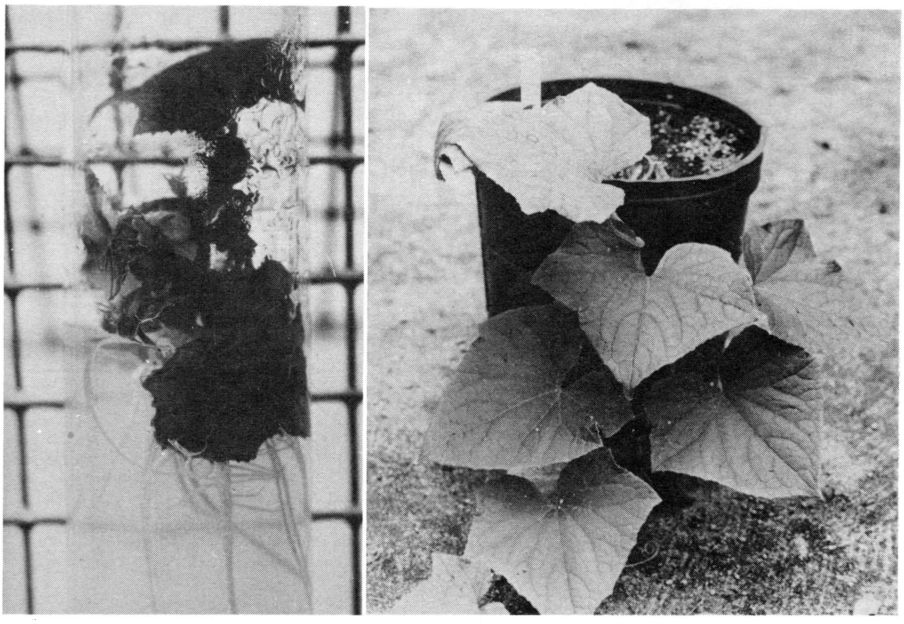

Fig. 1. Regeneration and proliferation of cucumber plants from axillary buds: L) 1 month old culture on 0.1 mg/liter kinetin and 0.1 mg/liter NAA; R) cucumber plant derived from tissue culture.

Axillary buds cultured in all 4 combinations of NAA and kinetin at 0.0 and 0.02 mg/liter grew very little, became extremely chlorotic, and eventually died (Table 1). Those buds placed in 0.5 mg/liter NAA callused over very rapidly and abundant root proliferation occurred regardless of kinetin concentration. The higher levels of kinetin (5.0-20.0 mg/liter) also induced a large amount of callus growth which completely overgrew the buds.

The highest percentage of plants produced was on the medium containing 0.1 mg/liter NAA and 0.1 mg/liter kinetin (Table 1). Only those treatments enclosed by dotted lines in Table 1 showed development of either roots or shoots. A large percentage of complete plants was produced on media containing 0.1 mg/liter NAA:0.1 mg/liter kinetin and 0.1 mg/liter NAA:0.02 mg/liter kinetin. The roots produced on the latter medium were few, long, and stocky. The roots produced on the 0.1 mg/liter NAA:0.1 mg/liter kinetin medium were more numerous, shorter, and more fibrous than those produced on other media. The best balance between root and shoot growth occurred on the 0.1 mg/liter NAA:0.1 mg/liter kinetin medium (Fig. 1) and the most plants were produced from this medium (Table 1).

An interrelationship between NAA and kinetin in plant regeneration and callus formation was observed. Auxin-free media containing moderate levels of kinetin (0.5-5.0 mg/liter) stimulated production of complete plants and callus production was minimal. With increasing levels of NAA, however, the level of kinetin necessary for plantlet production decreased. At the higher levels of kinetin, callus production took precedence over shoot growth.

Shoot growth of gynoecious cucumber plants *in vitro* was best obtained by a balance of both auxins and cytokinins. Cytokinin appeared to be necessary for the production of normal roots when NAA was at the 0.1 mg/liter level.

After 6 weeks incubation, rooted plantlets were successfully transferred to a mixture of equal parts soil, peat and perlite, with pH adjusted to 6.9 by the addition of lime. Transparent plastic cups were inverted over the pots for 1 week to ensure high humidity around the newly transplanted plants. Plants grown to maturity in an outside nursery retained the normal gynoecious character and appeared as the normal 'Carolina' type with respect to leaf and fruit morphology after 1 month (Fig. 1). Attempts to apply this procedure to propagate selections made in the field were hampered by a high rate of microbial contamination, not uncommon in field-grown (6) and greenhouse (8) plants. A more rigid surface-sterilization procedure was needed.

In vitro propagation of gynoecious cucumber plants using axillary buds offers a greater chance of obtaining a desirable selection from the field than does the traditional method of rooting cuttings. Since 5 to 6 axillary buds can be obtained from 1 cutting, bud culture has a 5-fold advantage.

Table 1. Production of complete plants (roots and shoot) from axillary bud explants after 4 weeks incubation on a culture medium (refer text).

Kinetin concn (mg/liter)	No. (and %) complete plants from explants: NAA concn (0.0 mg/liter)	(0.02 mg/liter)	(0.1 mg/liter)	(0.5 mg/liter)
0.0	0[z]	0[z]	8 (67)	0[y]
0.02	0[z]	0[z]	7 (58)	0
0.1	0 (0)	1 (8)	10 (83)	0
0.5	2 (17)	0 (0)	2 (17)	0
1.0	5 (42)	4 (33)	0 (0)	0
2.0	2 (17)	0 (0)	0 (0)	0
5.0	1 (8)	0 (0)	0	0
10.0	0 (0)	0 (0)	0	0
15.0	0 (0)	0	0	0
20.0	0 (0)	0	0	0

[z]Treatments to left of upper dotted line resulted in chlorotic buds.

[y]Treatments to right of lower dotted line resulted in heavy callus and root formation.

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